Non-lethal PCR genotyping of single Drosophila

Gil B. Carvalho, William W. Ja, and Seymour Benzer†
Division of Biology, California Institute of Technology, Pasadena, CA, USA

BioTechniques 46:312-314 (April 2009) doi 10.2144/000113088
Keywords: Drosophila; non-lethal; recombinant animals; molecular genotyping; stock lines

†Deceased

Supplementary protocol is available online at www.BioTechniques.com.

In Drosophila, genetic techniques relying on stochastic chromosomal rearrangements involve the generation and screening of a large number of fly stocks to isolate a few lines of interest. Here, we describe a PCR-based method allowing non-lethal molecular characterization of single flies. Using this procedure, individual candidate recombinant animals can be genotyped and selected one generation earlier than with extant methodology and, importantly, before stocks are established. This advance should significantly facilitate several of the most fundamental and routine techniques in Drosophila genetics.

Chromosomal recombination (CR) and mobilization of transposable elements (MTE) are two fundamental techniques in Drosophila genetics. CR is commonly used to associate or dissociate two alleles on different loci of a chromosome. MTE generates two classes of excisions: (*i*) precise (also called revertants), where the transposon is completely removed leaving a wild-type chromosome; and (*ii*) imprecise, where rearrangements occur to the transposon and/or the surrounding sequence (1). These methods have two major limitations. First, they rely on largely unpredictable chromosomal rearrangements. Hence, reliable characterization of the resulting DNA structure requires molecular genotyping, usually by polymerase chain reaction (PCR) and DNA sequencing. Second, these methods are generally inefficient. Since recombination frequency is proportional to the distance between the two loci of interest, achieving CR between two close loci requires screening a large number of candidate animals. Similarly, for MTE, isolating an imprecise excision causing a deletion in a given gene may require screening hundreds of lines, particularly when dealing with a large locus. These genetic experiments therefore typically involve generating single animals that bear unique chromosomal rearrangements, from which tens or even hundreds of individual stocks are derived and screened (1). Excisions isolated through a visible marker (such as eye color) and candidate recombinants can sometimes be screened phenotypically. However, phenotypes are often too burdensome for screening or unknown altogether (such as in the case of reverse genetics). In these cases, screening relies on molecular techniques. A procedure allowing non-lethal genotyping of single animals would allow direct molecular screening of first-generation candidate recombinants (i.e., at least one generation earlier than with classic methodology), with stocks established from only a few interesting individuals. Such a method should contribute to making CR and MTE efforts substantially faster, cheaper, and less burdensome. Here, we describe a PCR-based protocol for genotyping single Drosophila fruit flies using small body parts. Our technique allows DNA purification and amplification from the wings of a single, live fly with no significant impairment of robustness or reproductive ability.

DNA can be purified from a whole, single fly (2). We found that this protocol is also effective when using a single head or thorax, but not smaller body parts (Figure 1A and data not shown) and therefore requires the genotyped animal to be sacrificed. We developed a protocol allowing DNA purification from a pair of fly legs. The two forelegs of an anesthetized male were sectioned at the proximal femur, placed in a 0.2-mL PCR tube (Sorenson Bioscience, Salt Lake City, UT, USA) and covered with 10 µL of 400 µg/mL protease K (MP Biomedicals, Solon, OH, USA) in buffer A (10 mM Tris-Cl at pH 8.2, 1 mM EDTA, 25 mM NaCl). Optimal results were obtained when the biological sample was entirely submerged in the buffer. Importantly, homogenization was not necessary in these conditions and sufficient DNA was obtained by virtue of the protease digestion. The tubes were incubated at 37°C for 1 h and then at 95°C for 2 min to inactivate the protease. A 540-bp fragment of the Drosophila *actin* gene (*Act42A*) was amplified using the following PCR conditions: 1× iProof HF buffer (Bio-Rad, Hercules, CA, USA), 0.2 mM dNTPs, 0.5 µM primers (forward: 5′-GGTCGCGATTTAACCGACTACCTGAT-3′; reverse: 5′-CTCTTGCTTCGAGATCCACATCTGCT-3′), 3 µL fly leg DNA template and 0.4 U iProof High-fidelity DNA polymerase (Bio-Rad) in a total volume of 20 µL. Thermocycler conditions were 1 cycle of initial denaturation (98°C for 30 s); 35 cycles of denaturation (98°C for 10 s), annealing (63°C for 15 s), and extension (72°C for 20 s); and 1 cycle of final extension (72°C for 10 min). Running 5 µL of each reaction on an agarose gel revealed a single band of the expected size and similar in intensity to the product obtained from head DNA (Figure 1A, lanes 5 and 6).

Although this method isolated DNA suitable for PCR from a small body part, leg amputation is an invasive procedure that likely affects fitness. Since the goal of non-lethal genotyping is to select animals

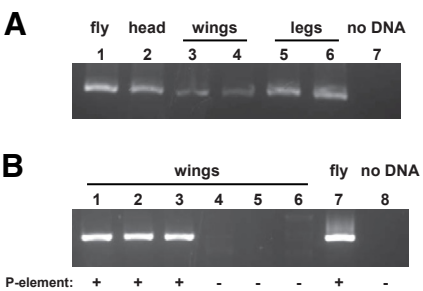

Figure 1. PCR amplification of DNA isolated from discrete body parts of a single fly. (A) DNA extracted by either the lethal purification method (see Reference 2) (lanes 1–2) or our non-lethal protocol (lanes 3–6) was PCR-amplified using primers flanking a 540-bp fragment of the *Act42A* gene. Biological samples: lane 1, whole fly; lane 2, single head; lanes 3–4, pair of wings; lanes 5–6, pair of legs; lane 7, no template. (B) DNA purified from wings of flies bearing the UAS-dSOD2 P-element insertion (lanes 1–3), but not control flies (lanes 4–6), allows PCR amplification of a product specific to the P-element. Lane 7, PCR from DNA extracted from a whole fly bearing the P-element. Lane 8, control reaction with no template added.

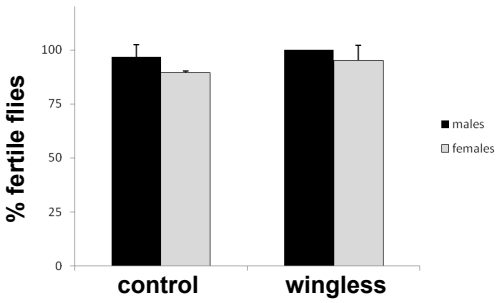

Figure 2. Wing ablation does not affect the fertility of males or females. Wingless or control flies were housed with one animal of the opposite sex in culture vials for 7 days and scored for viable progeny. *n* = 20–30 animals per group. Error bars, ±SD.

with which to establish a stock, flies must not only survive genotyping but also remain robust and fertile. We therefore proposed that fly wings could be used as the biological sample. Our online protocol successfully isolated enough DNA via a double wing ablation (a pair of fly wings, sectioned immediately distal to the wing base) to yield a visible band after PCR (Figure 1A, lanes 3 and 4). Increasing the concentration of protease was not beneficial, and shortening the incubation time reduced extraction efficiency (data not shown). As seen previously with leg-derived DNA, the results were most consistent when the wings were fully covered by the protease solution. Overall, using two wings for the extraction gave optimal reproducibility.

To test the specificity of the amplification from wing DNA, we PCR-amplified a sequence specific to the UAS-dSOD2 transgenic insertion (3) (the primers used were forward: 5′-AGTACTGTCCTCCGAGCGGA-3′ and reverse: 5′-TAGGGCAGCTTCGGTAGGGT-3′). A PCR product was obtained from DNA extracted from wings of UAS-dSOD2 flies, but not from controls lacking the transgene (Figure 1B).

We next investigated if double wing ablation affected reproductive ability. The courtship ritual of Drosophila males includes unilateral wing vibrations thought to influence female receptivity (4). We tested the ability of wingless flies to generate viable progeny when housed with a winged cohort of the opposite sex. The performance of wingless animals was indistinguishable from winged controls (Figure 2). This result demonstrates that double wing ablation does not affect long-term reproductive ability and constitutes a convenient non-lethal genotyping method.

A method for non-lethal genotyping has been described in the honeybee (5). The advent of a protocol designed specifically for non-lethal genotyping in Drosophila should significantly facilitate molecular genetics in this well-established genetic model organism. To a large extent, non-lethal PCR can replace current molecular methods in the context of fly genetics with a significant saving in time and cost, since it allows genotyping at least one generation earlier [i.e., before stocks are established (Figure 3)]. Candidate animals can be screened molecularly one by one or in small batches until a chromosomal event of interest is isolated, virtually eliminating unnecessary labor and reducing reagent costs. As with current methodology, the number of animals screened before interesting stocks are isolated varies from experiment to experiment. In practice, since a recombinant between two alleles located on different chromosomal

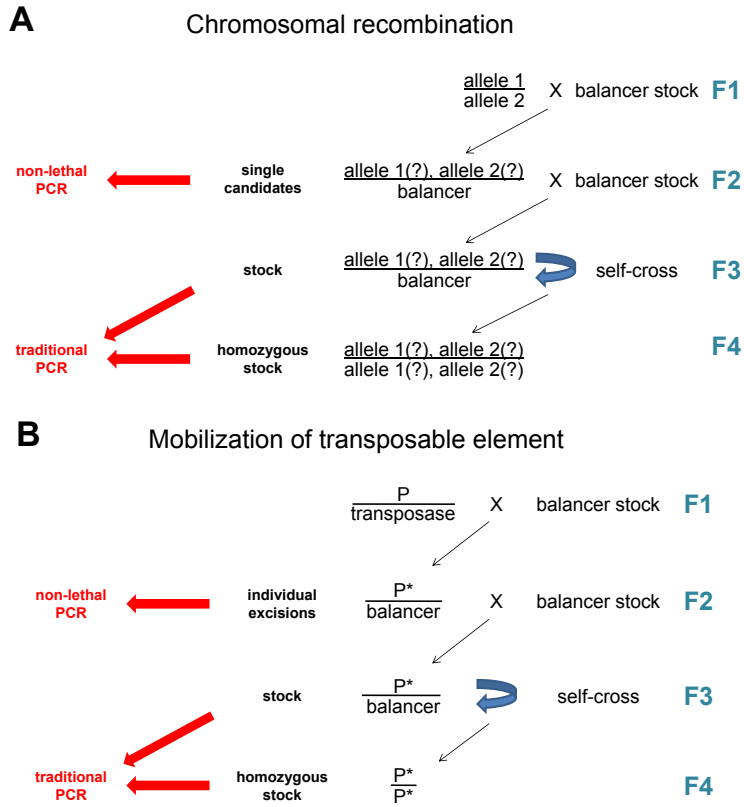

Figure 3. Non-lethal genotyping allows molecular characterization before stocks are established. (A) Chromosomal recombination. F1: individuals bearing the two alleles to be recombined in trans are crossed to a balancer stock. F2: candidate recombinants are isolated as individual female progeny and balanced to generate stocks. Non-lethal PCR allows genotyping at this stage. F3: balanced stocks of candidate recombinants are self-crossed to generate homozygous stocks. Traditional PCR allows genotyping at this stage or F4. F4: homozygous stocks. (B) Mobilization of transposable element. F1: flies bearing transposable element (P) in trans to a source of transposase are crossed to a balancer stock. F2: numerous individual animals bearing different excision events (P*) are isolated (typically on the basis of defective eye color) and balanced to generate stocks. Non-lethal PCR allows genotyping at this stage. F3: balanced excision stocks are homozygosed. Traditional PCR allows genotyping at this stage or F4. F4: homozygous excision stocks.

arms occurs, on average, 50% of the time, it often suffices to genotype two or three candidates to isolate such a line, with this number increasing as the distance between the alleles diminishes. As for MTE, the frequency of a particular excision event is a function of the properties of each transposon and its insertion site and ranges from very frequent (e.g., precise excisions of Piggybac elements, typically requiring only one or two candidates to be genotyped) to very infrequent (e.g., an imprecise excision resulting in a null allele of a large locus, which may require several hundred candidates to be screened).

Since first-generation recombinants bear the putative rearrangement—a recombination in CR and an excision in MTE—over a balancer chromosome, the PCR product should be unique to the chromosome of interest. Specifically, our method is useful in CR experiments to confirm the recombination of insertions of known sequence (by either using two primers complementary to the insertion or one on the insertion and another on the flanking genomic region) and deletions (using flanking primers). For MTE, useful primer sets include those with a primer on one end of the transposon and another on the neighboring genomic region, as well as primers flanking the insert, which can identify imprecise excisions where the size of the amplicon is reduced upon deletion of part of the endogenous chromosome.

Our protocol is particularly useful for identifying local transpositions, a specific case of MTE that takes advantage of the propensity of transposable elements to hop locally. The goal of local transposition is to mobilize an insert into a specific nearby gene of interest (6). Candidate animals can be effectively screened using a primer on one end of the insertion coupled with a set of primers in the target gene.

Note added in proof: It has been brought to the attention of the authors that a similar method has been previously described in a non–peer-reviewed, non-indexed publication by Gleason et al. [Gleason, J.M., K.A. Cropp, and R.S. Dewoody. 2004. DNA preparations from fly wings for molecular marker assisted crosses. Drosophila Info. Serv. *87*:107-108.]

Acknowledgments

We thank S.M. Lee and B. Zid for comments on the manuscript. This work was supported by funding from a Lawrence L. and Audrey W. Ferguson Fellowship (to G.B.C.), the National Institutes of Health (NIH; grant no. K99AG030493 to W.W.J. and grant nos. AG16630, AG24366, and DK070154 to S.B.), and the National Science Foundation (grant no. MCB-0418479 to S.B.). This paper is subject to the NIH Public Access Policy.

The authors declare no competing interests.

References

1. **Ashburner, M., K.G. Golic, and R.S. Hawley.** 2005. Drosophila: A Laboratory Handbook, 2nd ed. CSH Laboratory Press, Cold Spring Harbor, NY.
2. **Gloor, G.B., C.R. Preston, D.M. Johnson-Schlitz, N.A. Nassif, R.W. Phillis, W.K. Benz, H.M. Robertson, and W.R. Engels.** 1993. Type I repressors of P element mobility. Genetics *135*:81-95.
3. **Missirlis, F., J. Hu, K. Kirby, A.J. Hilliker, T.A. Rouault, and J.P. Phillips.** 2003. Compartment-specific protection of iron-sulfur proteins by superoxide dismutase. J. Biol. Chem. *278*:47365-47369.
4. **Kyriacou, C.P. and J.C. Hall.** 1986. Interspecific genetic control of courtship song production and reception in Drosophila. Science *232*:494-497.
5. **Châline, N., F.L.W. Ratnieks, N.E. Raine, N.S. Badcock, and T. Burke.** 2004. Non-lethal sampling of honey bee, *Apis mellifera*, DNA using wing tips. Apidologie *35*:311-318.
6. **Adams, M.D. and J.J. Sekelsky.** 2002. From sequence to phenotype: reverse genetics in *Drosophila melanogaster*. Nat. Rev. Genet. *3*:189-198.

Received 6 October 2008; accepted 17 December 2008.

Address correspondence to Gil Carvalho, California Institute of Technology, M/C 156-29, 1200 E. California Blvd., Pasadena, CA, 91125. e-mail: gil@caltech.edu